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isolated mitochondria. Accordingly, we hypothesized that downstream respiratory inhibition by sphingosine leads to increased formation of $O_2^{\bullet-}$ radicals after reperfusion, which by themselves have only a moderately harmful effect. However when Fe^{2+} redistributes from lysosomes into mitochondria during ischemia, Fenton chemistry occurs after reperfusion, leading to formation of highly reactive $OH^{\bullet}$ radicals, potent inducers of the mitochondrial permeability transition pore and cell death. This hypothesis was directly tested using bafilomycin, which induces the release of Fe^{2+} from lysosomes with subsequent uptake into mitochondria. Indeed, bafilomycin potentiated sphingosine-induced cell death. The data highlight a novel mechanism mediating I/R injury, which involves sphingosine accumulation and uptake of lysosomal iron into mitochondria during ischemia, leading to respiratory chain inhibition, iron-dependent oxidative stress, mitochondrial permeability transition and cell death after reperfusion. DK073336, DK037034 and 14.Z50.31.0028 (JJL) and NS083544 (TIG).

Cellular Signaling and Metabolic Networks

3085-Pos Board B515

Guardian Function of Mitsugumin 53 in Cell Membrane Repair and Metabolic Syndrome

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Mistugumin 53 (MG53) is an essential molecule in facilitating cellular membrane repair. A member of the TRIM protein family, MG53 possesses a RING domain functioning as an E3 ligase to mediate the down-regulation of insulin receptor substrate-1 (IRS-1) in insulin signaling. Conflicting results have been presented that elevated MG53 expression may serve as a causative factor for the development of metabolic syndrome. This study was designed to test whether high-fat diet (HFD) treatment alters the expression and function of MG53 within mice models of metabolic syndrome. Western blotting showed that MG53 expression does not change within the skeletal and cardiac muscles of mice subjected to HFD treatment. This data contradicts earlier findings presented by Song et al. (Nature, 494: 375-379, 2013), who claimed that MG53 expression is markedly elevated in animal models of insulin resistance and metabolic syndrome. Rather, we found that stress provoked by metabolic syndrome in the mice models actually reduced MG53 levels in the serum. Immunohistochemical analyses revealed that skeletal muscle fibers of HFD-induced mice experience localization of intracellular MG53 around mitochondria. The reduced MG53 serum levels observed may explain the compromised tissue regenerative capacity of diabetic patients. Clustering of MG53 around mitochondria may represent an adaptive response to metabolic stress. Overall, our study supports the guardian function of MG53 in cell membrane repair and metabolic syndrome. Therapeutic approaches for an elevation of MG53 expression in tissues that bypasses its interaction with IRS-1 may be a novel approach to treating human diseases with degenerative tissue repair functions.

3086-Pos Board B516

Non-Invasive Interogation of Signaling Activated Gene Regulation

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Many signal transduction and gene regulatory pathways are highly dynamic resulting in a variety of dynamic signaling and gene expression profiles. Currently, our biophysical understanding of these profiles relies on the manipulation of specific genes or drug treatment of specific proteins. One drawback of this approach is that a gene of interest needs to be identified to affect the pathways dynamic. Another drawback is that such perturbations may result in a significant interference with the function of the cell. In order to avoid these drawbacks, we developed a novel non-invasive perturbation approach to investigate the dynamic properties of signaling and gene regulatory pathways, without genetic manipulation or drug treatments. To demonstrate the feasibility of this approach we choose to interrogate a stress response pathway in yeast, which enables us to manipulate the intensity, duration and shape of the signal transduction profile. By combining quantitative single cell and single molecule experiments with predictive modeling, we are able to quantify signal transduction activation, signal transduction saturation and gene expression activation thresholds, which is not possible to quantify with any other currently available technology. We also found that the signaling dynamics is proportional to the first time derivative of the external perturbation profile. Because this approach is independent of the biological pathway or organism, it presents a general methodology to interrogate signaling and gene expression pathways non-invasively without the need for genetic or drug perturbations.

3087-Pos Board B517

Monte Carlo Simulation of Wnt Propagation by a Novel Transport Mechanism Complementing a Joint Experimental Study

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Tissue development is a key process in living organisms. An essential component for these developmental processes - but also for tissue regeneration and stem cell regulation - is the communication of cells by paracrine signaling. Following the French flag model, these processes are responsive to concentration gradients of signal carrying molecules, so-called morphogens. The highly conserved family of Wnt proteins can act as morphogens and represents important regulators of all these processes. After secretion, specific transport mechanism must ensure proper distribution of the morphogen. Experimental studies in zebrafish embryos and human kidney cells have given first evidence for a novel short-range transport of Wnt morphogens from the Wnt active tissue towards receiving cells using cell protrusions, so-called filopodia, as mediating agent. These specialized filopodia transmit signaling proteins between communicating cells and allow a high degree of control of propagation speed, direction and concentration of the transmitted ligand. The crucial question is how this novel short-range mechanism can result in a long-range gradient of morphogen molecules covering the complete responsive tissue. In order to give an answer to this question and address the theoretical feasibility of the new model we have set up complementary Monte Carlo simulations. The simulation iteratively reproduces ligand production, cell migration, and a slight ligand decay in concordance with experimentally measured boundary conditions. In a filopodia mediated transport system the major parameters are not anymore diffusion rate, cell adhesion, and concentration of the ligand but length, angle distribution, and growth frequency of filopodia. During the simulation we were able to identify key parameters of the underlying mechanism and quantitatively reproduce our experimental data. These results provide evidence that a filopodia based short-range transport system for Wnt has long-range signalling function.

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A Genetically-Encoded FRET Sensor based on AMP-Activated Protein Kinase Reports Allosteric Kinase Activation

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AMPK is a multi-protein nanomachine that is activated by multiple, complex mechanisms, allowing fine tuning of AMPK activity in different situations of metabolic stress. Binding of adenine nucleotides to the gamma subunit plays a major role in either direct allosteric activation of AMPK or modulation of AMPK phosphorylation and dephosphorylation by upstream kinases and phosphatases. These activation mechanisms require crosstalk between AMPK subunits by a nucleotide-induced conformational switch. We have engineered an AMPK complex that allows a direct, real-time readout of the AMPK conformational state by fluorescence energy transfer (FRET). This molecular sensor confirms the exquisite sensitivity of AMPK to low micromolar concentrations of AMP, shows the exclusive ability of ATP, but not MgATP, to compete with AMP, and allows insight into the role of CBS domains for allosteric AMPK activation. It has potential applications as a tool for screening of allosteric activators of AMPK, and as a reporter of cellular energy state.

3089-Pos Board B519

Integrated Omic Analysis of a Guinea Pig Model of Heart Failure and Sudden Cardiac Death

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Mechanistic understanding of heart failure (HF) and sudden cardiac death (SCD) has been hampered by the lack of suitable experimental models with features of human disease. We have developed a guinea pig model of cardiac hypertrophy (HYP) and HF, characterized by predisposition to SCD (Liu *et al.* Circ. Res. 2014). Our objective was to refine guinea pig models of HF progression by integrating protein, metabolite and transcript levels.
Relative protein abundances from sham-operated, HYP and HF hearts were assessed using isobaric tags for relative and absolute quantification, prior to liquid chromatography and tandem mass spectrometry (LC-MS/MS). Metabolites were quantified by LC-MS/MS or gas chromatography coupled to MS. Transcriptome profiles were obtained from Affymetrix microarrays.